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The work under the final portion of US Army grant DAMD17-96-1-6165 focused on the technical aspects to produce high density arrays in automated fashion. A printing robot was constructed that used a capillary print head developed in the first year of this grant. This printer was shown to produce arrays that performed as well as our previous hand-spotted arrays. Much less target DNA is necessary, permitting faster, cheaper automated clone purification procedures, such as the Autogen machine. Finally, I developed an improved narrow bore capillary print head capable of printing arrays of densities $10^4/cm^2$.

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Recommendations in Relation to the Statement of Work

For the second and final year of my postdoctoral work I developed an automated array printing robot (Figure 1) in collaboration with the Engineering Division at Lawrence Berkeley Laboratory (LBL). The capillary print head developed in the first year of this work was attached to a gantry linking a translation table holding four 864-well microtitre plates and an xy translation table holding 8x6 microscope slides. A good deal of time (1/98-4/98) was spent at LBL overseeing the assembly and working out printing the parameters (print head pressures, wash conditions, stage velocities, etc.) necessary to reliably print arrays in a high throughput manner. The robot is now incorporated into a core facility at the UCSF Cancer Center.

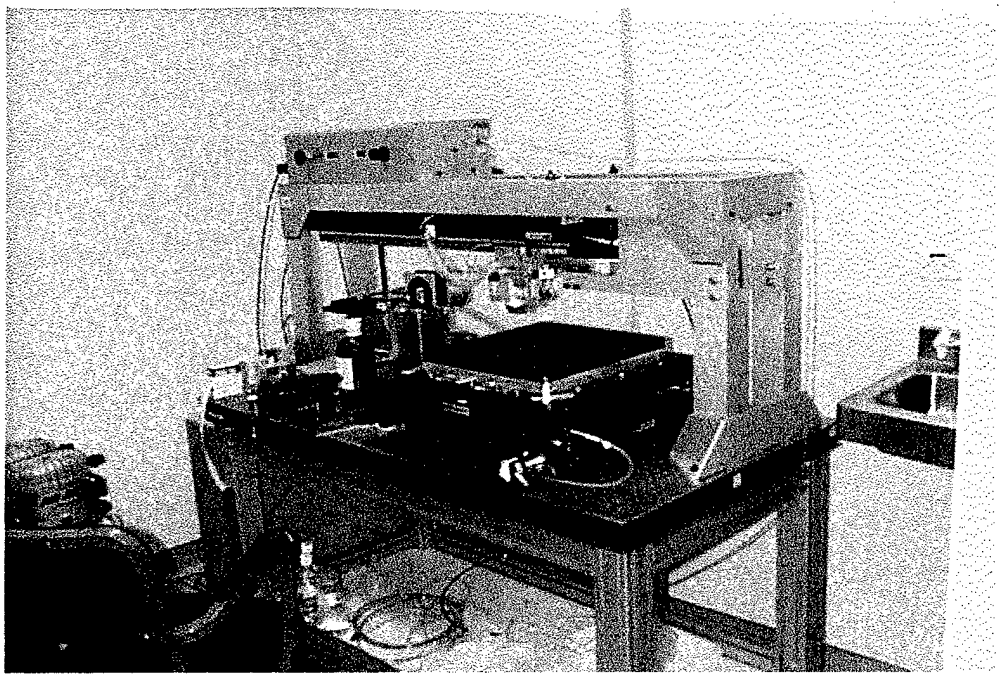

Figure 1. The array printing robot.

Method validation for printed arrays. It is possible that this printer may introduce artifacts, so results were compared to previous results obtained from manual capillary printed arrays. An array was prepared

using 23 clones spaced along chromosome 20 (the 'chromosome 20 scanning array' mentioned in the previous report) and probe was prepared from the cell line BT474. Results were in excellent agreement (data not shown) indicating that the printing process did not introduce artifacts.

Automation of the array fabrication process. Large genome scanning arrays (~3,000 clones, resolution 1 Mbp) are planed for preparation in the upcoming years, so automated methods for clone production were evaluated. We obtained an Autogen machine which is capable of preparing ~180 clones from 1.5 ml bacterial cultures in a ten hour period. These clones were dissolved in our nitrocellulose/DMSO target solution at 1 ug/ul and compared with clones prepared using manual Quiagen columns (~6-12 clones per day from 100-500 ml cultures). The Autogen spots were quite weaker in the DAPI (counterstain) channel than the Quiagen clones were. A comparison of absorption, fluorescence, nuclease digestion and gel data for these clones indicated that the Autogen preps contained a significant amount of contaminating low molecular weight nucleic acids. Nonetheless, clones prepared in these two ways displayed identical fluorescence ratios. (data not shown)

Defining the minimum amount of target DNA placed on the arrays. From above discussion, it is clear that more targets can be prepared in a more rapid , inexpensive manner if lower DNA target concentrations are used. Also, the printing process is expected to be more reproducible for the current 2 ug/ul BAC or P1 target solutions are quite viscous. Calculations and 'double spotting' experiments indicate that only the top layer of DNA is available for hybridization. To test how little DNA was needed in the target solution a dilution series of lambda DNA was printed on a slide and hybridized with 50, 500 or 5,000 pg of FITCand

Texas Red labeled lambda probe. these experiments showed that the fluorescence ratios did not change from 2-0.06 ug/ul of target DNA concentration (data not shown). The spot shapes, however, did change, with the lower concentrations showing a 'coffee stain' appearance, an effect due to lessed visscocity during drying.

Production of dense arrays. The print head I developed (described in detail in the previous Progress Report) uses 375 um o.d. fused silica capillaries to deliver the target solution to the slide. I showed that by choosing smaller inside diameters, such as 25 um, extremely dense ($10^4/cm^2$) arrays can be printed. However, such narrow bore posses extremely high hydrodynamic impedance (9-fold higher that the currently used 75 um i.d. capillaries) making their use slow during the loading and washing steps. It is for these reasons that I developed a dual bore capillary, which has a short length of 25 um i.d. 150 um o.d. capillary glued inside a 4 cm length of 375 um o.d. 150 um i.d. capillary. These capillaries have not been tested yet, however, they are expected to produce dense arrays in a more rapid, reproducible manner.

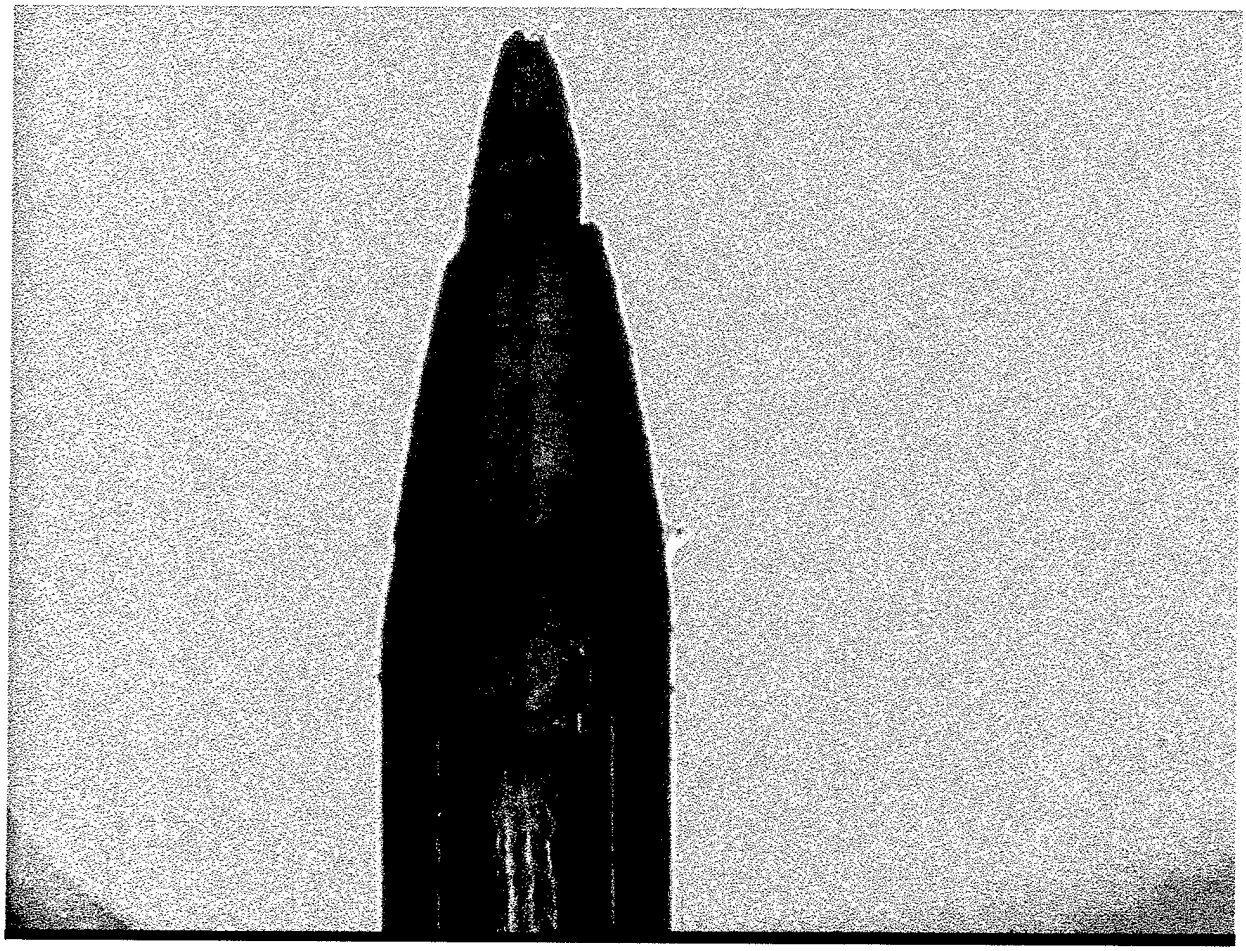

Figure 2. Closeup of the dual bore capillary consisting of a 0.4 mm long 25 um i.d. section glued into a 4 cm long 150 um i.d. 375 um o.d. capillary.

Conclusions

The work under the final portion of US Army grant DAMD17-96-1-6165 focused on the technical aspects to produce high density arrays in automated fashion. A printing robot was constructed that used a capillary print head developed in the first year of this grant. This printer was shown to produce arrays that performed as well as our previous hand-spotted arrays. Much less target DNA is necessary, permitting faster, cheaper automated clone purification procedures, such as the Autogen machine. Finally, I developed an improved narrow bore capillary print head capable of printing arrays of densities $10^4/cm^2$.